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SAMPLING AND ANALYSIS OF CONTAMINATED SUBSISTENCE TRAILERS

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NOTICES

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Contamination of commissary subsistence became manifest when ingestion of packaged food items resulted in prolonged organic aftertaste, nausea, and vomiting. This report describes the sampling and analysis procedures (cryogenic trap and grab air sampling) used to isolate and identify the contaminating species. The principal contaminating agent was ethylidene norbornene (ENB), which had been coshipped with the subsistence vans.

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SAMPLING AND ANALYSIS OF CONTAMINATED SUBSISTENCE TRAILERS

INTRODUCTION

During a 4-month period beginning October 1972, approximately 70 cargo vans containing brand-name resalable (BNR) commissary subsistence arrived at several U.S. Army and Air Force installations in western Europe in a highly contaminated condition. The vans were sea/land trailers, 40 feet long by 8 feet wide by 8 feet tall (Fig. 1). They each had a payload volume of about 2500 cubic feet and carried a mixed cargo of foodstuffs, laundry, personal hygiene, and baby-care items; the cargo value of each container ranged from $15,000 to $30,000. An odor within the vans indicated a chemical contaminating agent(s). The problem became manifest when customers returned packaged food items to the commissaries, complaining of organic aftertaste, nausea, and vomiting following ingestion.

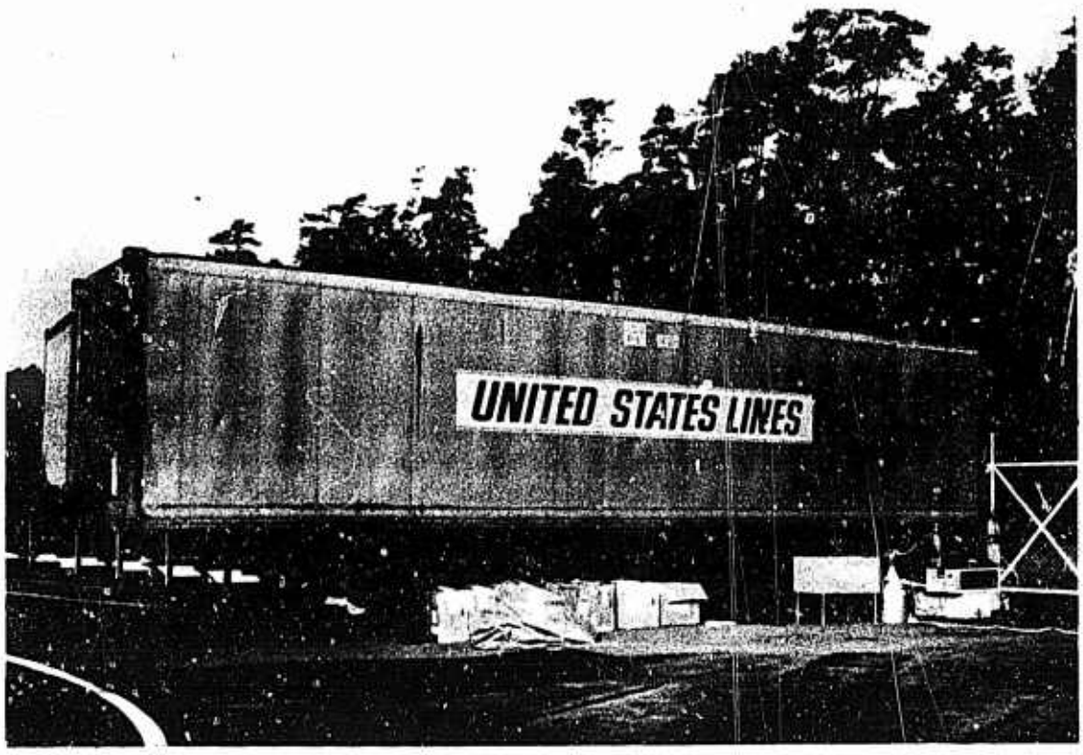

Figure 1. Typical sea/land trailer used to transport BNR subsistence.

In November 1972, following preliminary unsuccessful efforts by local laboratories, the Clinical Medicine Branch, Hq USAFE, asked the USAF School of Aerospace Medicine (USAFSAM) for analytical assistance in identifying the contaminating agent(s).

In response to this request the Bioenvironmental Analysis Branch, USAFSAM, conducted cryogenic trap, grab container, and thin-layer chromatographic (TLC) plate sampling at Ramstein Air Base, Germany on two separate occasions. During 21 November - 8 December 1972, 9 cryogenic trap samples were taken from four contaminated vans full of BNR nonperishable subsistence. Two 30-liter pressurized grab air samples and several packages of disposable diapers were also taken from two of the contaminated vans. Contaminated food vans reappeared after a 6- to 8-week lull, and the second sampling exercise was conducted 9-24 January 1973; 5 cryogenic trap samples were drawn--3 from contaminated refrigerated vans, and 2 from uncontaminated vans containing BNR non-perishable subsistence. The latter served as background samples to help isolate the contaminant compound.

This report describes the sampling and analytical procedures used to isolate and define the contaminating species. The principal agent was found to be the organic compound ethylidene norbornene (ENB), which, it was later ascertained, had been coshipped with the subsistence vans. Of approximately 90 compounds identified, 52 were common, in varied concentrations, to both contaminated and noncontaminated trailers. Ethylidene norbornene, however, was the only compound observed in all samples taken from contaminated vans, and not in any from the contaminant-free trailers. This compound, which appeared in relatively high concentrations, was identified by a combination of chemical analysis and information exchange. Mass spectrometry was used to initially classify the compound as a bicyclic heptene homolog. After norbornene was suggested as a possible identity, it was determined that a similar chemical, ethylidene norbornene, had been coshipped with the contaminated subsistence containers. Mass spectrometry analysis of pure ENB samples from three sources then verified that the unknown compound was identical to ENB. Ethylidene norbornene is a bicyclic monomer that contains two double bonds and is used as an intermediate in the manufacture of ethylene propylene-diene-monomer (EPDM) rubber.

SAMPLING

Several types of samples were taken to detect, identify, and confirm the contaminating agents. These included:

a. cryogenic trap samples of van air
b. pressurized grab samples of van air
c. contaminated disposable diapers
d. thin-layer chromatographic plate exposures

e. methylene chloride washings of van walls
f. liquid samples of ethylidene norbornene.

Cryogenic Trap Samples

A total of 14 cryogenic trap air samples were taken--9 during the first sampling trip and 5 during the second--to concentrate organic constituents from a large volume of air drawn from the vans, above and around the contaminated cargo. Table 1 lists the sampling schedule, and Figure 2 shows the cryogenic trapping system on station. Cryotrapping operates on the principle that a gaseous compound will condense (and therefore collect) at a temperature where its vapor pressure is less than its partial pressure (concentration) in the sample stream (3). Acetone, for example, which has a vapor pressure of approximately 10^{-8} mm Hg at -175°C, will condense in a -175°C trap when acetone in the sample stream is greater than about 0.01 ppb.

TABLE 1. CRYOGENIC SAMPLING SCHEDULE, RAMSTEIN AB, GERMANY

Date	Trailer No.	Sample No.	Sampling time (min)	Total sampled (liters)	Ambient temp range (°C)
24 Nov 72	USLU 4153453	1	360	180	1 to 4
25 Nov 72	USLU 4153453	2	818	409	0 to 8
26 Nov 72	USLU 4005607	3	802	401	-5 to 5
27 Nov 72	USLU 4005607	4	730	365	-3 to 2
29 Nov 72	USLU 4174844	5	810	405	-1 to 5
30 Nov 72	USLU 4174844	6	810	405	3 to 7
1 Dec 72	CT1U 2918274	7	720	360	3 to 8
2 Dec 72	CT1U 2918274	8	810	405	5 to 11
5 Dec 72	USLU 4153453	9	720	360	7 to 10
16 Jan 73	USLU 2021321	10	720	360	2 to 7
17 Jan 73	S/L 56677	11	720	360	1 to 6
18 Jan 73	USLU 7145430	12	720	360	1 to 3
19 Jan 73	USLU 3983430	13	720	360	-1 to 4
20 Jan 73	USLU 3983430	14	720	360	0 to 4

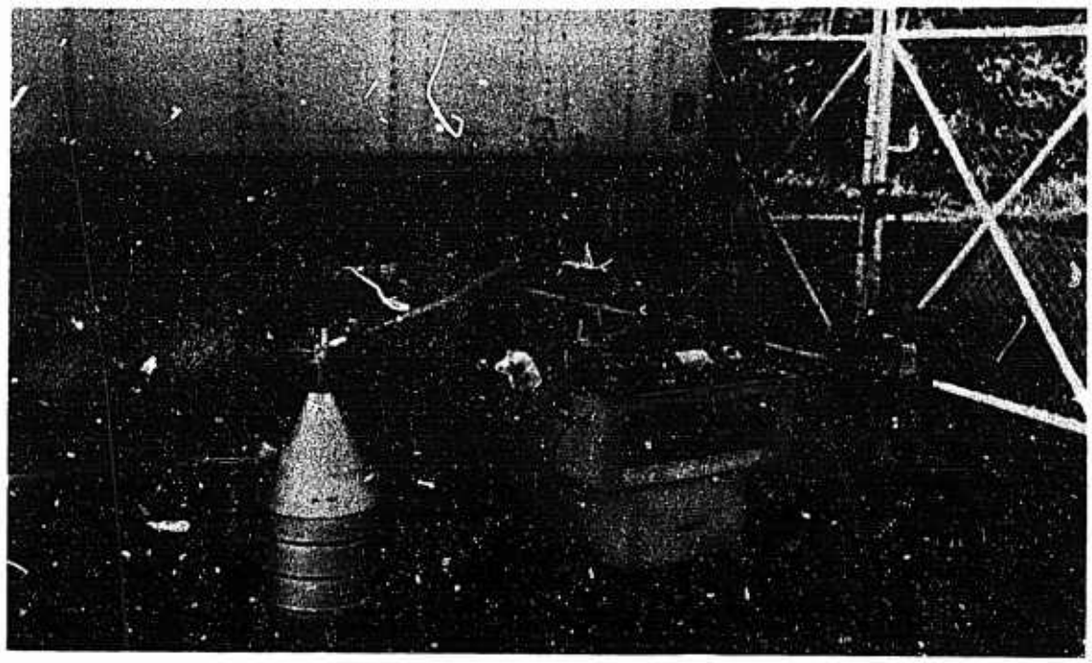

Figure 2. Cryogenic trapping system sampling air space within subsistence trailer.

The USAFSAM cryogenic trapping system (shown systematically in Fig. 3) used three cold traps in series maintained at (1) 0°C--ice water, (2) -78°C--dry ice, and (3) -175°C--liquid nitrogen. Since liquid nitrogen was not available during our first sampling trip, liquid oxygen (giving a final trap-condensation temperature of about -160°C) was used as the cooling medium in the third trap for the first 9 samples collected. The air sampling rate was 500 ml/min, drawn through each trap in series by a metal bellows pump. The sampling line was a 5-m length of 6.4-mm O.D. Teflon tubing. The sample intake was inserted about 2 meters into the van with the door almost entirely closed. The sampling time for each van varied from 6 to 13.5 hours, with an average of 12.1 hours. Average total air volume sampled for each van was 370 liters, corrected to 760 mm Hg and 21.1°C.

The trap-collecting vessels were 150-ml-capacity, stainless steel cylinders (Whitey P/N HDF4-150-304). Three cylinders formed one sample set. Each set was packed in crushed dry ice for shipment to an analytical laboratory at either Monrovia, California or Brooks AFB, Texas. The sample (set) taken 2 Dec 72 from van CTIU 2918274 was a controlled, documented sample and followed chain-of-custody procedures. This sample was hand-carried by AF custodian from point-of-collection at Ramstein AB, Germany to point-of-analysis at Analytical Research Laboratories (ARL), Monrovia, California.

GAS FLOW PATH FOR MULTISTAGE CRYOGENIC
TRAPPING SYSTEM

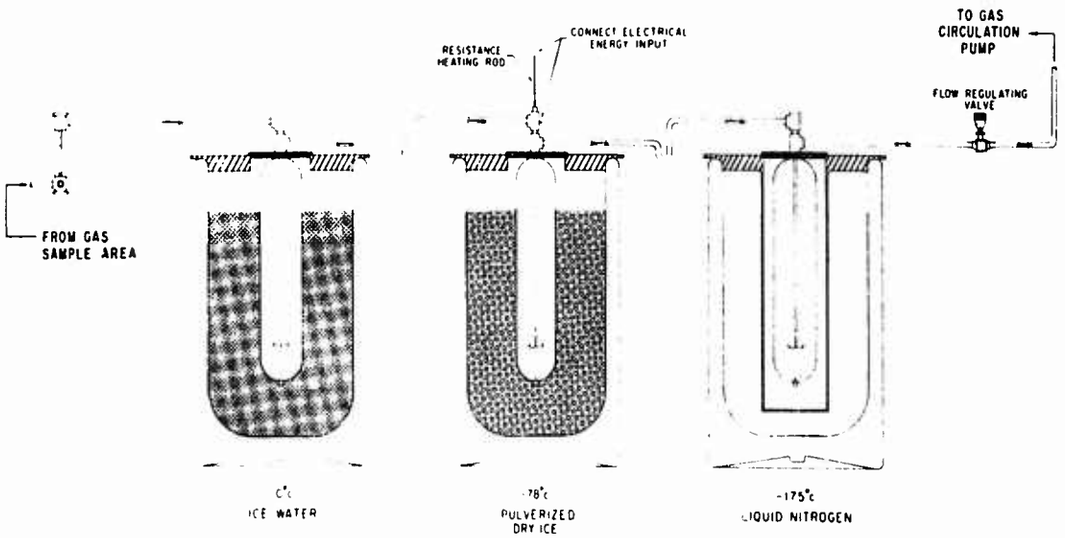

Figure 3. Schematic diagram of USAFSAM cryogenic trapping system.

Grab Air Samples

Two grab air samples were taken from contaminated vans in 35-liter oxygen cylinders. The cylinders were pressurized to about 35 psig with metal bellows pump.

Other Samples

Thin-layer chromatographic plates were deployed in two vans in an attempt to detect the presence of moderate- to high-molecular-weight organic compounds; i.e., 16-18 carbon atoms or greater. The plates were 8-in-square glass coated on one side with silica-gel absorption matrix. Twenty plates were exposed for 12 to 48 hours. The plates were packed in a sealed container (with Pampers) for shipment.

Van walls were washed with methylene chloride in an attempt to detect contaminant adsorption/penetration into the interior plywood/metal surfaces; 4-in-square gauze pads presoaked in methylene chloride were used. The pads were subsequently packed in a sealed metal can for shipment.

Disposable diapers were selected for off-gas analysis because of their highly absorptive characteristic and hence their potential to magnify the contaminating agent. Four boxes of diapers were taken from a contaminated van and packed in a sealed metal container for shipment.

Liquid samples of the suspected contaminating agent, ethylidene norbornene, were obtained later in the investigation from three sources--B.F. Goodrich Co., Orange, Texas; Union Carbide Chemicals and Plastics Division, South Charleston, West Virginia; and a Union Carbide European Subsidiary in Belgium, via the U.S. Army. The liquid samples of ENB were shipped by parcel post in unpressurized, sealed glass bottles.

ANALYSIS AND RESULTS

The bulk of the cryogenic trap analyses, as well as the disposable diaper, TLC-plate, washing, and ENB analyses, were done by Analytical Research Laboratories, Monrovia, California under Air Force contract AF41609-72-C-0034 (1, 2). The grab air samples and one cryogenic trap sample set were analyzed at the USAFSAM Bioenvironmental Analysis Branch, which also conducted confirmatory assays on the ENB samples.

Cryogenic Trap Samples

Fourteen sets of cryogenically trapped gas samples were analyzed; 13 by ARL and 1 by USAFSAM. Each set consisted of materials trapped at $0^{o}C$, $-78^{o}C$, and either $-160^{o}C$ (liquid oxygen) or $-175^{o}C$ (liquid nitrogen). Two sets were background samples from uncontaminated vans, and 12 sets were from six contaminated vans including two refrigerated trailers. The ARL sample sets were analyzed by gas chromatography and mass spectrometry (GC-MS), using a previously reported standard procedure (6) which is summarized in Appendix A.

Table 2 shows the results of the cryogenic trap analyses conducted by ARL. All values are reduced to milligrams of compound per cubic meter of sampled air and listed by compound type.

Of the compounds detected, only ENB appeared in all of the contaminated trailer samples and not in either background sample. Finding this initially unknown compound in relatively high concentration made it an early suspect and prompted a concerted effort to identify it. Other compounds which appeared in most of the contaminated trailers and not in the background (or very low in the background) included Freon-11, Freon-113, chloroform, tetrachloroethylene, toluene, cumene, methyl alcohol, ethyl alcohol, and limonene. The observed concentration of these compounds was in every case far less than their accepted

threshold limit value (12) and in most cases below their known odor threshold (10). This fact tended to discount the implication of any of these compounds as the primary causative agent.

Compounds identified by mass spectrometry that were not properly identified by retention volume alone were methyl isopropyl ether, pinene, and propyl and butyl benzenes. A few identities for low-level components may be questionable but were listed because the compounds noted (chloropropene, hydroxyquinoline, and ethylene glycol) did match the retention volumes and detector response. Crotonaldehyde was a tenuous identification because it was not fully resolved from toluene on the carbowax column, and the concentration of toluene was generally so large that the presence of a poorly resolved compound could not be readily determined. The unknown compounds at the end of Table 2 were listed by column and/or detector, and time of elution. The identification of two compounds as pinene and limonene suggested that several of the unknowns may be close-boiling isomers such as camphene, dipentene, and terpinene, all of which have molecular weight 136.2.

Concentration checks between duplicate sample sets were acceptable. In each pair, the more highly contaminated sample was apparently the result of a higher ambient temperature during that sample-collection period. The high-contaminant levels found in samples 7 and 8 from trailer CTIU 2918274, confirmed the exceptionally malodorous nature of this trailer noted by the sampling crew. The background trailers did not differ greatly from the contaminated trailers either in total contaminant content or in number of compounds detected.

Figure 4 shows the USAFSAM analysis of cryogenic trap sample 14, done by GC-MS procedure detailed in Appendix B. This sample was taken from a contaminated refrigerated trailer containing frozen meat products. The figure shows the mass spectrometer total ion count (ordinate) as a function of scan number (abscissa) for each of the three traps. The lower curve represents the 100% relative ion count, and the upper curve a 5-fold magnification showing 20% as full scale. The peaks were identified by computer analysis of individual scan fragmentation patterns and comparison with known spectra contained in the computer library. Qualitatively, these data confirmed that the cryotrap apparatus operated according to design. The major recovery of contaminants was in the -175°C trap, with only a modest amount at -78°C (10%-20%) and very little at 0°C (5%). This analysis also correlated reasonably well with sample 13 (Table 2), taken earlier from the same trailer, showing relatively high concentrations of dichloromethane (methylene chloride), methanol, ethanol, n-pentane, trichloroethylene, and ethylidene norbornene.

TABLE 2. ANALYSIS OF CRYOGENICALLY TRAPPED AIR-SPACE SAMPLES FROM BNR SUBSISTENCE TRAILERS (mg/m^3)

Trailer:	USLU 4005607		USLU 4153455			USLU 4174844		CTIU 2918274		USLU 7145430[a]	USLU 3983430[a]	USLU Sea-Land 2021321[b]	USLU Sea-Land 56677[b]
Sample:	3	4	2	1	Floor 9	5	6	7	8[c]	12	13	10	11
Freon 11	.034	.070	.001	.005	.039	.021	.018	.025	.12	t[d]			
Freon 12	t		t	.002	.002	t	t	.23	.49	t	.073	t	.076
Freon 113	t				.001	t	.030	t	t		.21		
Freon 114					.004		t						
Chloroform	.016	.011	.037	.045	1.3	.001	.043	.048	.16	t	.007		.01
Methylene chloride	.11	.013	.031	.032	.10	.10	.13	.049	.28	.36	.37	.014	.65
Carbon tetrachloride											.052		
Methyl chloride	.002	t	.004	.006	.001								
1,1,1-Trichloroethane	.032	.018	.001	.002	.030	.023	.018	1.3	1.6	.064	.070	.001	.035
1,2-Dichloroethane										.074	t	t	t
Trichloroethylene	.013	.005	.017	.029	.45	.089	.071	.32	.42	.084	.38	.049	.27
Tetrachloroethylene					.15		.24	.19	.010			.012	
Ethyl chloride	.001	t	t	t					.003	.022	t		
Vinylidene chloride		t			.005								
Vinyl chloride	.041		t										
3-Chloropropene	.003		.001	.002		.009	.034	.010					
Dichlorobenzene							t		.002		t		t
Propane	t	t	t	.001	.002	t	t	.18	.025	.001	t	.004	.002
Butane	.004	t	.001	.001	.003	.008	.039	.71	1.9	.004	.005	.001	.003
Isobutane	.001	t	t	t			.067						
Pentane										.001	.2		.002
2,2-Dimethyl butane							.012				.033		
Hexane									.30				
Trimethyl hexane										.032	.002	.034	.008
Propylene	t		t	t	t		t		.55	t	t	.001	.001
1-Butene	.001	t	.001	.001	.001	.002	.026	.001	1.1	.004	.004	.016	.078
2-Butene							.002					t	t
Isoprene										.01	.20	.004	.066
2-Hexene	t	t						t	t	.002		t	
1,3-Butadiene							.004			t	t	.001	.002
Cyclohexene	.001	t	.001	t							.022		
Methyl cyclohexene					.002	.007	.003	.001	.003	.036	.014	.022	

Methyl cyclohexane		.001	t					.041	.33	.039		.007	
Methyl cyclopentane					.007	.006	.010			.008	.010	.013	.007
Benzene	.027	.007	.007	.013	.018	.013	.015	.14	.12	.035	.037	.014	.011
Toluene	.52	.23	.10	.10	.38	.081	.015	.21	.34	.11	.076	.028	.020
m-Xylene										.061	.021		
o-Xylene										.014			
p-Xylene										t			
Ethyl benzene	.049	.011	.001	t	.009	.035	.001	.12	.004		.059	.029	.04
C9 aromatics	.011		t							.031	.014	.015	.017
Trimethyl benzene	.015	t	.041	.058	.20		.006	.032	.032	.025	.008	.008	.007
Mesitylene	.003			t			.001	.034	t		.005	.002	.016
Indene										.003	.009	.001	t
n-Propyl benzene	.015	.002	.027	.024	.10	.012	.020			.006	.009	.017	.016
Cumene	.020	.01	.053	.009	.20	.009	.041	.17	.060	.035	.010	.007	.006
C10 aromatics	.004	t	.003	.074				.019		.004		.004	.002
C11 aromatics					.08				.008				.004
Furan		.007	.006				.015		.001	.002	.004	.008	
Dioxane	.003	.004	.003	t	.082	.014	.008	.006	.003	.042	.029	.020	.01
Methyl furan			.001		.016	.071			.14				
Methyl alcohol	.23	.085	.17	.048	.22	.58	.45	.53	.91	1.8	.15	.073	.004
Ethyl alcohol	.18	.036	.089	.11	.23	.25	.29	.24	.40	.17	.057	.067	.037
Ethylene glycol					.13		t[d]	.004					.015
Allyl alcohol					.003		.016	.055	.085				
n-Propyl alcohol	.001	t	.006	.003	.16	.008	.007	t	.008	.11	.031	.01	.002
Isopropyl alcohol	.015	.003	.073	.030	.62	.13	.18	.14	.22	.088	.25	.031	.029
T-butyl alcohol							t	.008	.031	.027	t	.004	
n-Butyl alcohol													.013
IsoButyl alcohol			.006	t					.22	.067	.001	.011	.028
n-Amyl alcohol					.40			.005		.004	t	.007	
IsoAmyl alcohol											.019		
Cyclohexyl alcohol										.003		.008	t
Capryl alcohol								t		.003		.004	t

[a]Refrigerated trailer.
[b]Clean trailer (background).
[c]Documental chain-of-custody sample.
[d]Signifies less than 0.001 mg/m^3.

TABLE 2. (continued)

Trailer:	USLU 4005607		USLU 4153455			USLU 4174844		CTIU 2918274		USLU 7145430[a]	USLU 3983430[a]	USLU 2021321[b]	Sea-Land 56677[b]
Sample:	3	4	2	1	Floor 9	5	6	7	8[c]	12	13	10	11
Hydroxyquinoline	.054	.001	.009										
Acetone	.070	.017	.029	.019	.69	.097	.13	.33	.94	.090	.220	.061	.042
Methyl ethyl ketone	.027	.008	.005	.007	.022	.018	.028	.017	.038	.029	.005	.005	
Methyl Isobutyl ketone	t[d]	t	.027	.016									
Diethyl ether										.001		.016	.022
Methyl Isopropyl ether	.037	.006	.18	.016		.005	.004						
Acetaldehyde	.018	.002	.018		.58	.081	.10	1.2	.004		.057	.013	-
Crotonaldehyde	.002		.001	.018	.001	t	.001	.001	t				
Benzaldehyde				t	.005								
Methyl acetate					.083								
Ethyl acetate	.009	.002	.005	.002	.023	.033	.045	.23	.59			.051	.10
Propyl acetate									.38				
Butyl acetate	.004	.001	.005				.011		.13	.007	.013		.001
Nitromethane			t		.001								
Pinene					.60	.053	.027	.034	.14				
T-butyl benzene					.24	.037	.006	.088	.089				
Methylal					.008	.023	.023	.005	.007	t	.003		
Unknown EC-C-1925	.001	t	.011	.006	.14		t	.011	t				
Unknown EC-C-1960	t	.002	.001	.004									
Unknown Q-2625	.003	.001	.001	.001	.008	.011	.003	.032	.11	.003			.009
Unknown EC-Q-2750	t	t	.001		.004						.004		
Unknown C-1800					.026	.008	.005	.025	.017				
Unknown C-1280		.003			.005								
Ethylidene norbornene	.19	.011	.58	.75	1.5	.24	.26	.48	.39	.058	.09		
Limonene	.046	.003	.30	.40	.57	.082	.050	.36	.24				
TOTAL	1.81	.57	1.86	1.83	8.26	2.15	2.50	7.63	12.93	3.54	2.88	.75	1.72

[a]Refrigerated trailer.

[b]Clean trailer (background).

[c]Documental chain-of-custody sample

[d]Signifies less than 0.001 mg/m^3.

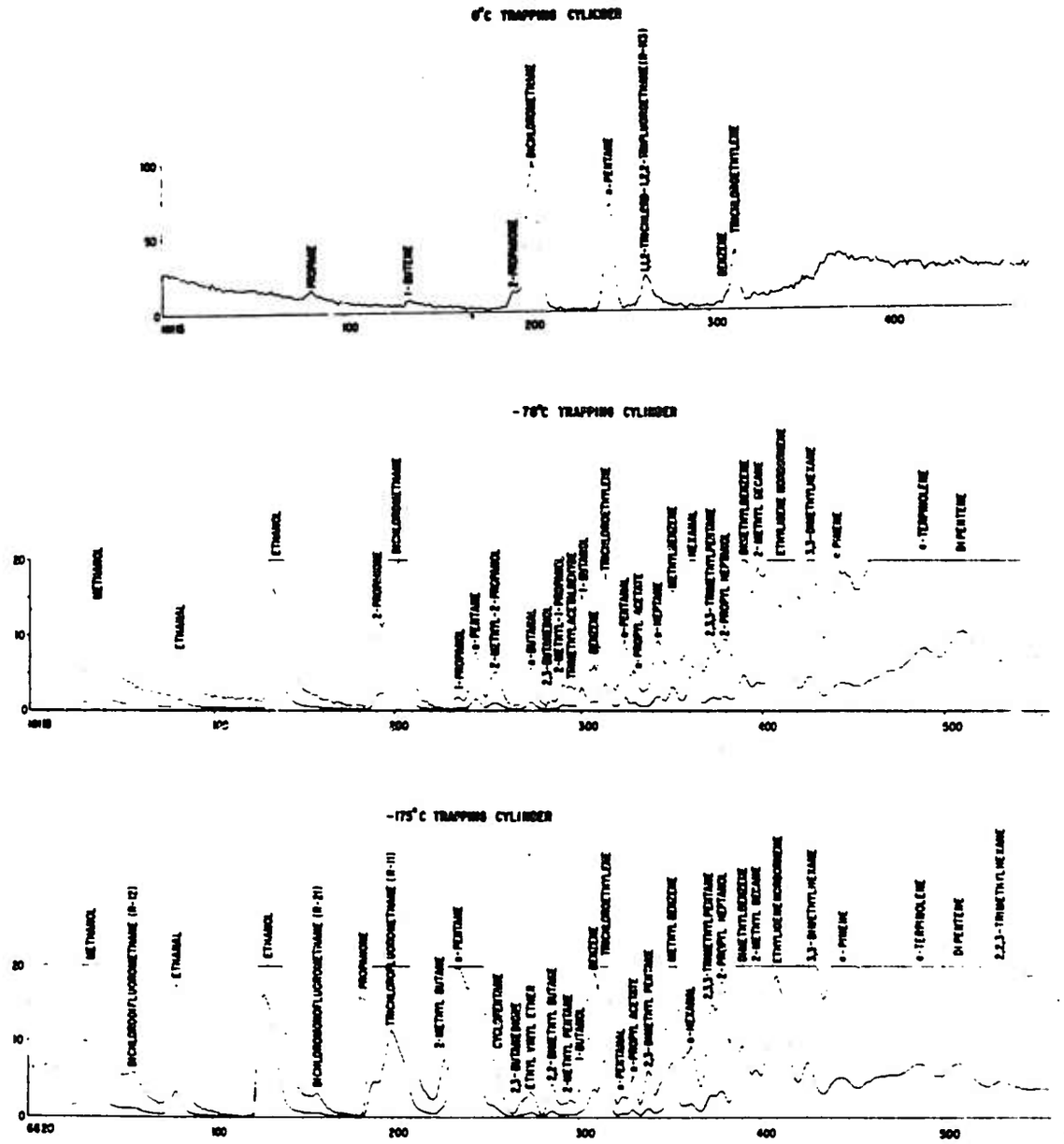

Figure 4. Mass chromatogram of cryogenic trap sample 14 (trailer USLU 3983430). Analysis conducted on USAFSAM GC-MS system.

Grab Air Samples (USAFSAM)

The grab air samples were analyzed by two methods; the first was chromatographic analysis of the cylinder gas to verify the quantitative estimates obtained from the cryotrap analyses. The chromatograph was a single-column instrument (Beckman Model GC-5) using helium ionization detection. The column was a 0.6-m length of 3.2-mm O.D. thin-walled stainless steel tubing, packed with 80-100 mesh Porapak Q. The carrier gas was helium at a flow rate of 12 cc/min. The chromatographic analysis was temperature programmed; 4-min isothermal at 40°C, 8°C/min to 167°C, and isothermal at 167°C to completion (about 30 minutes total).

Table 3 shows quantitative results for toluene on the two grab samples taken from trailers USLU 4005607 and 4153435. The objective of this assay was to verify that the average concentrations obtained by cryogenic sample (Table 2) were approximately correct and not in error by one or several orders of magnitude. These data indicated a somewhat higher toluene content in the two vans than was determined by cryogenic trapping. Since toluene was about midway in the boiling range for the total contaminant spectrum, it may be assumed, based on this analysis, that the overall trapping efficiency was about 30%-60%. In fact, the efficiency was probably lower for the lower molecular weight components and greater for the heavier constituents. The concentration decay noted in successive toluene assays was apparently due to loss of pressure in the sample cylinders.

TABLE 3. TOLUENE ANALYSES OF GRAB AIR SAMPLES (USAFSAM)

	Peak area (mm^2)	Concentration (mg/m^3)
Toluene Standard	1,000,500	192 (50 ppm)
Trailer No. USLU 4005607	9240	1.77
	7253	1.39
	4166	.80
Trailer No. USLU 4153435	1482	.28
	968	.19
	874	.17

The second method of grab air analysis involved contaminant concentration to facilitate detecting and identifying unknown/unresolved species. Contaminants in the grab air tank were concentrated by eluting sample at a flow rate of 160 cc/min for 20 minutes through a 2.9-cc chromatographic sample loop contained in a liquid nitrogen bath (-196°C). To vent the carbon dioxide trapped by this procedure

and to retain the organic constituents, the loop was warmed to -60°C in a cryocool unit and opened momentarily to ambient. The loop was then connected into the GC-MS system and analyzed in the same manner as the cryogenic trap samples.

Figure 5 shows the GC-MS mass chromatogram of the two grab air samples. The results correlated reasonably well, in terms of major constituents, with their cryotrap-sample counterparts in Table 2. Both samples indicated substantial relative concentrations of ENB. The identification of each peak was based on a "similarity" index of between 725 and 950. This index is an absolute measure of the degree of match between an unknown and a particular reference spectrum (7). Past experience has shown that a similarity index of 725 or better will usually permit greater than 95% confidence that the identification is correct.

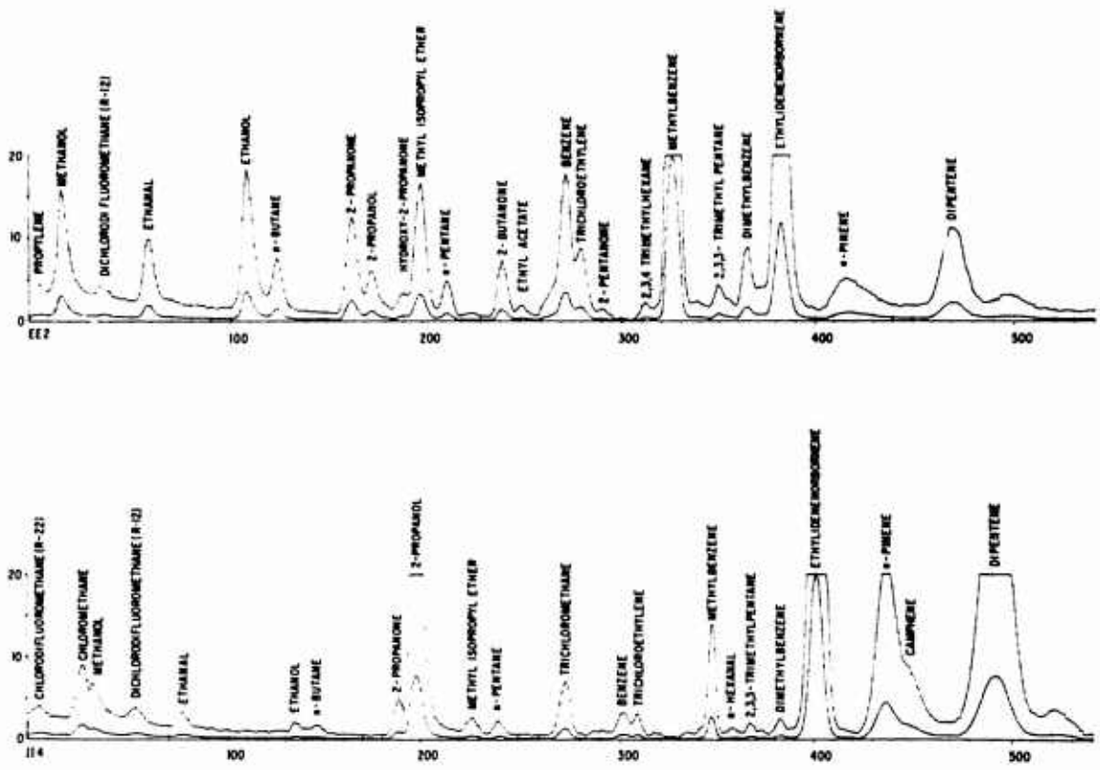

Figure 5. Mass chromatogram of cryogenically concentrated grab air samples, taken from trailers USLU 4005607 (EE2) and 4153435 (114).

Disposable Diapers

A disposable diaper from the shipping drum was placed in a round-bottom flask and desorbed on a glass vacuum rack at 75°C for 1 hour. For comparison, a disposable diaper of the same brand from a locally purchased box was similarly desorbed. The desorbates were split in the same manner as the cryotraps and analyzed by gas chromatography. Table 4 shows the contaminants so desorbed from the two samples. This analysis confirmed the presence of ENB in the trailer specimen and also indicated a greatly magnified total contaminant loading. The contaminated disposable diapers were, in fact, the most malodorous of all the collected samples.

TABLE 4. ANALYSIS OF OFF-GASSING OF DISPOSABLE DIAPERS

	Contaminated (µg)	Fresh (µg)
Methylene chloride	.02	
1,1,1-Trichloroethane	.24	
Vinylidene chloride	.06	.01
Methyl cyclopentane	.11	t
Methyl cyclohexane		.16
Benzene	.28	
Toluene		1.1
Xylene		.19
n-Propyl benzene	.03	
Mesitylene		t
Cumene	.01	
Indene		.01
Furan	.04	
Methyl alcohol	4.8	
Ethyl alcohol	8.5	2.4
Isopropyl alcohol	6.7	1.8
Isobutyl alcohol	.36	
n-Amyl alcohol	.03	
Hydroxyquinoline	.01	
Acetone	.53	1.3
Methyl isobutyl ketone	.57	t
Ethylidene norbornene	.48	
Limonene	1.2	.29
T-butyl benzene	1.3	
Pinene	.01	
TOTAL	25.30	7.36

Other Analyses

The TLC silica-gel plate scrapings and the gauze pads used to wipe the trailer were both extracted with methylene chloride, and the extracted material was subjected to infrared analysis. Neither spectrum showed any absorption peaks that could be associated with ethylidene norbornene. If this compound were present in the extract, its concentration was below the detection threshold. The spectrum from the gauze pads showed an appreciable quantity of a carbonyl compound, possibly a polyether ester. Such compounds are used as sizing materials, antistatic agents, and filler compounds.

IDENTIFICATION OF ENB

ENB was initially identified on the basis of its mass spectra, which gave a parent peak (molecular weight) of 120 atomic mass units (amu) and a maximum mass-to-charge (m/e) signal at 66 amu. A study of several mass spectral indexes (4, 5, 8, 11) did not provide positive identification, but suggested a bicyclic heptene compound of the norbornene type. The mass spectral data on ethylidene norbornene itself was not contained in any of the reference indexes. Other possible identifications considered at the time were phenyl derivatives or an unresolved mixture of two or more compounds including isopropyl benzene and norbornene.

For lack of a positive identification, these several possibilities were forwarded to the USAF and USA representatives in Germany. Subsequent investigations revealed that a structurally similar chemical, ethylidene norbornene, had been coshipped, in bulk tanks, with the contaminated trailers.

Three samples of ENB were then obtained for confirmatory analysis by both ARL and USAFSAM. In all samples, the odor of pure ENB was identical to (although much stronger than) that of the contaminated grab air samples and disposable diapers. Because of the extremely penetrating odor, the liquid ENB samples at ARL were transferred to small stoppered serum vials for GC analysis. Small quantities of ENB were withdrawn from the vial and introduced into the chromatograph in one of two ways: vaporization in the gas sample inlet, or direct injection through a silicone septum. No noticeable differences in GC elution time or mass spectrometer fragmentation pattern were found between the two methods of sample introduction. Fragmentation patterns of the ENB were also obtained by direct injection of vapor taken from the bulk-sample container.

Table 5 shows the mass spectra of the 3 pure ENB samples and 1 ENB peak from chromatographic separation of a cryotrap sample conducted by ARL; pure ENB was run on a CEC Model 21-130 mass spectrometer, and

the GC-separated ENB on a CEC Model 21-104. The fracture patterns for the 3 pure ENB samples were virtually identical; sample 1 had a distinctly higher m/e 78 peak and a slightly higher m/e 51 peak, which were characteristic of the slightly higher benzene impurity of this sample. The small differences noted in the fracture pattern of the cryotrap ENB sample 4 were due in part to the different instruments and instrument conditions used and in part to the fact that the lower m/e mass peaks were not recorded (27, 39, and 41 amu). Injection of the pure samples into the 130 instrument was done from a batch inlet system at an ionizing source temperature of 250°C; whereas the GC inlet was continuous through a sample enrichment system at a source temperature of 200°C.

TABLE 5. RELATIVE INTENSITY OF MASS PEAKS OF ETHYLIDENE NORBORNENE (ARL)

m/e	ENB[a]	ENB[b]	ENB[c]	ENB[d]
27	.23	.23	.23	
39	.32	.31	.31	
41	.13	.13	.13	
51	.13	.11	.11	.08
65	.10	.10	.12	
66	1.00	1.00	1.00	1.00
67	.09	.08	.09	
77	.14	.13	.13	.17
78	.31	.24	.23	.25
79	.13	.13	.13	.18
91	.41	.42	.40	.54
92	.09	.10	.09	.17
105	.40	.41	.40	.44
120	.20	.21	.19	.28

[a]Pure (97%) ENB from B.F. Goodrich Co., Orange, Tex.
[b]Pure ENB from Union Carbide Co., Charleston, W.Va.
[c]Pure ENB from European Tire Manufacturer, Belgium.
[d]ENB peak from cryotrap sample 2.

At USAFSAM, ENB was run on the GC-MS system primarily to obtain a mass spectrometer fragmentation pattern to identify and verify unknown peaks in the van-air analyses. Since our library did not contain ENB, we ran this species on the same instrument under the same conditions as the unknowns. Vapor samples of ENB were injected directly into the GC-MS. The fragmentation pattern obtained was inserted into the computer library and used to identify the ENB in previously analyzed cryogenic trap and grab air samples.

Table 6 shows the USAFSAM mass spectra obtained on two pure specimens of ENB and three ENB peaks from contained cryotrap and grab air samples. The data showed normal scatter, confirming that the spectra were of the same compound. The primary peak was uniformly at m/e 66, with strong secondary peaks at m/e 39, 91, 105, 78, and 120. While the order of these fragments was not uniform in all samples, the appearance of these peaks supports the molecular structure of ENB and stable fragments (Fig. 6).

TABLE 6. RELATIVE INTENSITY OF MASS PEAKS OF ETHYLIDENE NORBORNENE (USAFSAM)

m/e	ENB[a]	ENB[b]	ENB[c]	ENB[d]	ENB[e]
27	.59	.42	.24	.11	.14
39	.73	.71	.37	.19	.19
41	.13	.23	.22	.10	.12
50	.05	.12	.06	.05	.05
51	.11	.18	.17	.11	.10
52	.25	.12	.06	.06	.05
53	.06	.13	.12	.09	.07
65	.11	.21	.21	.12	.14
66	1.00	1.00	1.00	1.00	1.00
77	.29	.23	.29	.15	.13
78	.38	.41	.33	.29	.22
79	.09	.18	.28	.17	.14
91	.64	.59	.59	.64	.40
92	.25	.19	.24	.13	.15
105	.44	.53	.57	.71	.31
120	.29	.28	.40	.26	.24

[a]Pure (97%) ENB from B.F. Goodrich Co., Orange, Tx.
[b]Pure ENB from Union Carbide Inc., Charleston, W. Va.
[c]ENB peak from cryotrap sample 14.
[d]ENB peak from grab sample G1 (USLU 4153435).
[e]ENB peak from grab sample G2 (USLU 4005607).

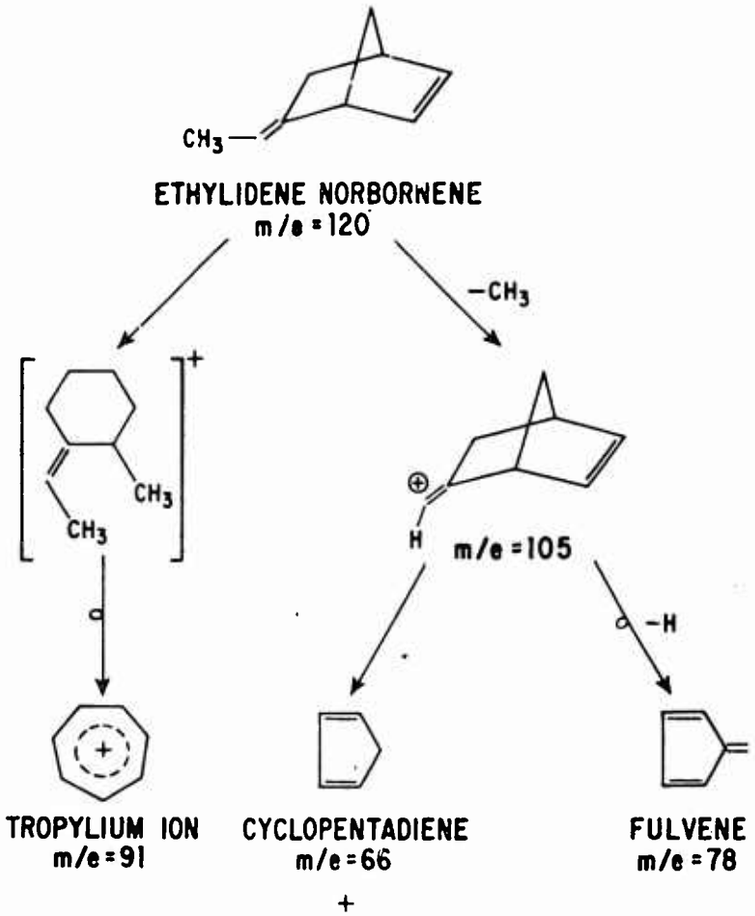

Figure 6. Chemical structure of ENB, and proposed pathways to stable ions resulting from mass spectrometer fragmentation.

DISCUSSION AND CONCLUSIONS

Based on the analytical findings presented here and the coincident discovery of the coshipped ENB, there is little doubt that the primary contaminant agent was one compound, and that that compound was ethylidene norbornene. A total of 89 specific compounds were detected in one or more air/specimen samples from the contaminated trailers;

52 of these were also detected in the background samples. Only the following 10 compounds appeared in a majority of contaminated trailers and in a concentration significantly different from background: trichloroethane, tetrachloroethylene, toluene, methanol, ethanol, cumene, acetaldehyde, crotonaldehyde, limonene, and ENB. Any of these compounds may have been attributable to specific cargo items in the vans, but the fact that only ENB was entirely unique to all contaminated vans was of itself strong circumstantial evidence.

The cause and effect relationship between ENB and the observed symptomology is beyond the scope of this report, and largely unknown. The standing concentration of ENB in contaminated vans varied from 0.01 to 1.5 mg/m^3 as determined by cryogenic trapping. When one assumes a 50% trapping efficiency, this range increased from 0.01 to 3.0 mg/m^3. This analysis, however, represents only a residual concentration of the trailer air space. The actual ENB content of packaged food items could have been significantly greater since exposure continued for several days and the source was (presumably) essentially pure ENB.

Kinkead et al. (9) found ENB moderately toxic to animals when it was inhaled or ingested; the concentration for 50% mortality (LC-50) varied from 732 ppm of the vapor for female mice to 3100 ppm for male rabbits. The same authors reported the odor threshold of ENB, as determined by a panel of 6 human subjects, to be 0.014 ppm (0.4 mg/m^3). The current (1972) threshold limit value for ENB is 0.6 mg/m^3 (12), which is lower than that of any other single compound found in the vans. Hence the known toxicity supports the conclusion that ENB was the primary contaminating agent.

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APPENDIX A

CRYOGENIC TRAP ANALYSIS PROCEDURE (ARL)

Each cylinder in the set was attached, in turn, to a special all-metal, heated, vacuum system and expanded into three stainless steel, valved, 75-cc bottles. The trap-sample cylinder was then heated and diluted by flushing with small increments of helium until an overall pressure of 10 psig was achieved. The system was allowed to equilibrate for 15-20 minutes before the sample valves were closed and the split bottles removed.

The split samples were analyzed by gas chromatography and mass spectroscopy. The chromatograph (F & M Model 5756B) was a dual-column instrument with both flame ionization and electron capture detectors. The dual columns were connected to two 8-port valves in such a manner that one column foreflushed while the second backflushed. The column packings were Porapak Q and Carbowax 1000. Porapak Q, a cross-linked ethylvinylbenzene-divinylbenzene polymer, gave excellent separation for compounds boiling below 100°C; Carbowax 1000 (polyethylene glycol) provided separation for compounds in the boiling range from 50°C to 240°C (e.g., acetone through methylnapthalene). The overlap range provided a verification of identity based on elution time. The Porapak Q column (100-120 mesh) was a 3.6-m (12-ft) length of 3.2-mm (1/8-in) O.D. thin-walled stainless steel tubing. The carbowax column was 10% loading of polyethylene glycol on Gas-Chrom Q (100-120 mesh), packed in a 7.3-m (24-ft) length of 3.2-mm thin-walled stainless steel tubing.

Chromatographic analyses were conducted separately on each column (i.e., separate split bottles). The operating parameters were the same for each column. The carrier gas was helium at a flow rate of 50 cc/min. The chromatographic run was time-temperature programmed; 6-min isothermal at 30°C, 6°C/min to 150°C, and 20-min terminal isothermal at 150°C. The overall chromatogram required 60 minutes per column run, and 20 minutes were allowed between consecutive runs to insure cooling of the column to the same starting temperature.

The chromatographic column effluent was split to provide for flame ionization and electron capture (EC) detection, with a third fraction directed to the mass spectrometer (CEC Model 21-104). The flame ionization detector was used for hydrocarbon detection, and the EC for halogenated compounds. The flame ionization and EC signals were recorded on a 1-mv dual-channel recorder (Mosely) with automatic signal attenuation. The flame signal was also fed, in parallel, to a digital integrator. The recorder trace was used to establish compound elution time and to verify the integrator printout. For most of the

compounds, identification was made by elution-time (or retention volume, V_R) comparison with known materials. ARL has accumulated a catalog of V_R values for over 250 compounds on the two analytical columns, using identical procedures.

The mass spectrometer was used as a qualitative tool to verify compound identity and, when signal concentrations were sufficient, to identify occasional unknown GC peaks by (the compound) fragmentation pattern. The column effluent fraction directed to the mass spectrometer was passed through a Watson-Biemann separator to provide a 5-fold enrichment of the sample.

To determine any unrecovered contaminants following chromatographic analyses, selected cryogenic-sample cylinders were rinsed with methylene chloride; the rinsings were analyzed by both gas chromatography and infrared spectrophotometry (Perkin-Elmer Model 237B).

APPENDIX B

CRYOGENIC TRAP ANALYSIS PROCEDURE (USAFSAM)

USAFSAM analyzed 1 cryogenic trap sample, using a coupled GC-MS system (Dupont Model 21-491) equipped with a dedicated data analysis subsystem (Dupont Model 21-094). The GC was a single-column instrument (Varian Model 1400) with a flame ionization detector (FID). The column was a 1.8-m (6-ft) length of 3.2-mm (1/8-in) O.D. thin-walled stainless steel tubing packed with 80-100 mesh Porapak Q. The carrier gas was helium at a flow rate of 30 cc/min.

The cryogenic trap cylinders were analyzed individually by heating to 100°C and expanding into an evacuated sample loop (2.9-cc volume), which was in turn flushed into the chromatograph. The GC-run was initiated at 0°C and temperature programmed at 4°C/min from injection to 150°C, and at 8°C/min from 150° to 245°C. The final temperature was maintained for 30 minutes to allow all compounds to elute. The GC column effluent was split into two streams; one-fourth going to the GC-FID and three-fourths to the mass spectrometer via a jet-type helium separator. Continuous mass spectrometer scans of the GC effluent were made at 5-sec intervals. Each scan covered the mass range from 219 to 12 amu and was made at the rate of 2 sec/decade. The total ion count for each scan was stored in the data processor and later used to generate a printed record of the chromatographic run. The fragmentation patterns obtained in individual scans were analyzed by the data processor and library search program (7) to determine the most probable identity of each eluting compound (chromatogram peak).